PROTOCOL

NANOPULSE SEBORRHEIC KERATOSIS STUDY

(NP-SK-002)

March 17, 2017
Rev. 4/17/17
Rev. 4/28/17
Rev. 7/14/17

CONFIDENTIAL

BACKGROUND

The Pulse TX is a medical device intended to clear the skin of benign, undesired skin lesions as an alternative to surgery and other more ablative methods for removing benign lesions. The system is designed to deliver a timed series of very low energy, high voltage (lesser than the voltage of static electricity) pulses of a time length between 100 and 750 nanoseconds (billionths) of a second. The non-thermal effect on tissue takes place in a very shallow depth of skin directly below the sterile treatment tip. The device emits significantly less energy than existing laser, electro-surgery or electro-cautery equipment.

Extensive *in vitro* research demonstrates a stimulation of a programmed cell death called apoptosis in a treated area, which is one of the presumed mechanisms for observations of clinical and histologic changes to cells in multiple studies of *in-vivo* treated tissue. In animal studies with over 1000 rats and mice, nano-pulsed devices have been demonstrated to reduce or eliminate benign tumors with a clinically acceptable margin of safety. A pilot clinical trial was conducted under IRB oversight in 2012 with a similar version of the Pulse TX device, with the findings published in *Experimental Dermatology.* (See Attachment A) A total of ten basal cell carcinomas on the skin of three Subjects were treated with a range of settings. Seven of the ten lesions were free of basal cells at the conclusion of the study, two partially resolved, and one recurred. No scars were visible at the healed sites of any of the successfully treated lesions.

PRIOR CLINICAL INVESTIGATIONS

Study Name:	NanoPulse Human Tissue Study
Study Number:	NP-ABD-001
Device Named:	Pulse TX System
PI Name:	David Kaufman, MD
Co PI:	Michele Martinez, RN
Study Dates:	November 2016 to January 2017

A total of seven subjects participated in this prospective, single site, non-randomized study. All of the participants were females representing several Fitzpatrick Skin Tone Classifications, and ranging from the low 40's to middle 60's in age.

The study was comprised of two sequential segments. The first segment called for the serialized use of the test device in specific locations on intact abdominal skin predetermined to be resected during a standard abdominoplasty. The primary endpoint of the first segment of the study was to establish that the trauma to the tested skin would be minimal. The appearance of each intact tested tissue location was comparable to that anticipated following any minor surgical insult.

Lidocaine was injected prior to use of the Pulse TX skin exposure. Subjects reported little or no sensation during the treatment session. Subjects further reported little or no discomfort after the Lidocaine effect had diminished. There were no adverse events, no reports of bleeding, and no scarring noted by the investigator at the end of the 60 day study.

The second segment of the study called for the histological analysis of tissue samples prepared from the abdominal section that were resected at the time of the abdominoplasty. Internal and outside expert histologists were consulted. This second segment did not impact the participating subject. Consequently, Pulse Biosciences, Inc. secured IRB oversight concentrated on the first segment of the study. It should be noted however that the histological analysis to date confirms that the Pulse TX was successful in achieving its intended effect. See Attachment B for a Summary Histological Report.

PROPOSED SEBORRHEIC KERATOSIS STUDY

Study Name: Nanopulse Seborrheic Keratosis Study
Study Number: NP-SK-002
Device Named: Pulse TX System
PI Name: David Kaufman, MD
PI Name: James Newman, MD
PI Name: TBD
Study Dates: TBD

PURPOSE AND SCOPE OF THE STUDY

The primary purpose of the study is to evaluate the use of the Pulse TX System when used to remove Seborrheic Keratosis (SK) lesions from the non-facial areas of healthy adult subjects. These benign skin lesions are frequently located on the limbs and/or trunk and not considered to be pre-cancerous. Patients routinely seek their removal for cosmetic/aesthetic reasons. SK lesions are typically treated using cryogen or a scalpel, or dessication with a

heat-based energy technology such as a laser or electrosurgery. SKs do not self-resolve without treatment.

STUDY OBJECTIVES

The specific objectives of the study are as follows:

- Document the non-treated appearance of off-face SK's
- Evaluate the clearance of SK's in off-face locations after removal using the Pulse TX System vs. pretreated appearance of the same lesion
- Evaluate the clearance of the treated SK's at various points in time over several weeks following the initial procedure, compared to the pre-treated SK's

PRIMARY ENDPOINT

- The primary endpoint of the study is the degree of clearing of benign SK lesions as rated by the independent reviewer's assessment of photographs of the lesions at the final study visit.

  - A 4-point scale (0, 1, 2, 3) will be used wherein each lesion will be characterized as "Clear", "Mostly Clear", "Partially Clear", "Not Clear".

Photographic Review of SK Lesions Rating Scale			
Clear	**Mostly Clear**	**Partially Clear**	**Not Clear**
0	1	2	3

  - See Attachment C for a protocol specific to the Independent Review.

SECONDARY ENDPOINTS

- A secondary endpoint of the study is the degree of clearing by the final visit as rated by the treating physician's assessment.

  - The same 4-point scale will be used as that used for the Photographic Review.

Investigator Assessment of SK Lesions Rating Scale			
Clear	**Mostly Clear**	**Partially Clear**	**Not**

			Clear
0	1	2	3

- An additional secondary endpoint of the study is subject satisfaction with the SK's area at the final study visit. A 5-point scale (0, 1, 2, 3 4) will be used wherein the Subject will be asked to rate their level of satisfaction with each SK area as "Satisfied", "Mostly Satisfied", "Partially Satisfied", "Dissatisfied" Highly Dissatisfied".

Patient Satisfaction for each SK area at day 106				
Satisfied	**Mostly Satisfied**	**Partially Satisfied**	**Dissatisfied**	**Highly Dissatisfied (worse)**
0	**1**	**2**	**3**	**4**

SUCCESS CRITERIA

- Independent Reviewer Assessment of Photograph: A score of "0" or "1" at the last follow-up will represent a successful outcome for that lesion. The study will be considered a success if 65% of treated lesions are reduced by a score of 2 or more points on the lesion scale
- Physician Assessment: A score of "0" or "1" at the last follow-up will represent a successful outcome for that lesion. Success for this criteria is 65% of lesions rated as a 0 or 1 on the scale.
- Patient Assessment: A score of "0" or "1" at the last follow-up will represent a successful outcome for that lesion. Success for this criteria will be 65% of lesion areas evaluated as "Satisfied" or "Mostly Satisfied".
- The overall study will be considered a success if 2 of the 3 criteria listed above are met.

STUDY DESIGN

A prospective, randomized, open label, multi-site, NSR study design where each subject serves as his or her own control will be employed. A Principle Investigator will be identified at each of up to five participating sites. A total of up to 60 Subjects will be consented and enrolled with anticipated enrollment expected to be uniformly distributed across sites. The PI will consider the study inclusion and exclusion criteria and invite appropriate patients to participate in the study.

No significant risk is posed by either the use of the Pulse TX System or the study process. While safety can never be taken lightly, this study is not meant to measure, monitor or analyze any significant disease or disorder for which medical treatment is mandatory. (See additional information regarding Non-Significant Risk statement in the following pages.)

ANALYTICAL METHODS

Three sets of data will be analyzed:

1. Clinical observational data as derived from the examinations.
2. Data captured during the photograph review process.
3. Patient self-reported symptoms and observational data.

The statistical analysis for this single-arm NSR study will be performed after collection of safety, effectiveness and secondary outcomes during the baseline period and each evaluation interval.

- Analysis of primary endpoints in intent-to-treat population
- Analysis of primary and secondary endpoints in the per protocol population
- The calculation of the change from baseline in the treated lesion size and appearance will be adjusted by the observed effect on the untreated lesion change in size and appearance
- Exploratory Data Analysis to describe distributions and to explore trending
- Repeated measures ANOVA or non-parametric equivalent to compare means
- Likelihood ratio statistics to assess categorical data
- Chi-Square Tests to compare binary proportions
- Exact probabilities will be computed for any inferential analysis performed
- 95% confidence levels will be calculated using exact methods
- Agreement statistics to estimate the inter- and intra-photographic reviewer variability
- All analysis will be adjusted for the correlated nature of the data since more than one lesion is being sampled from the host subject

All tables and listings will be produced and stored in SAS datasets. No data imputations will be performed.

SAMPLE SIZE ESTIMATE

A one group χ^2 test with a 0.050 two-sided significance level will have 91% power to detect the difference between the Null hypothesis proportion π_0, of 0.510 (51%) and the Alternative proportion, π_A, of 0.650 (65%) when the sample size is 135.

DEVICE DESCRIPTION

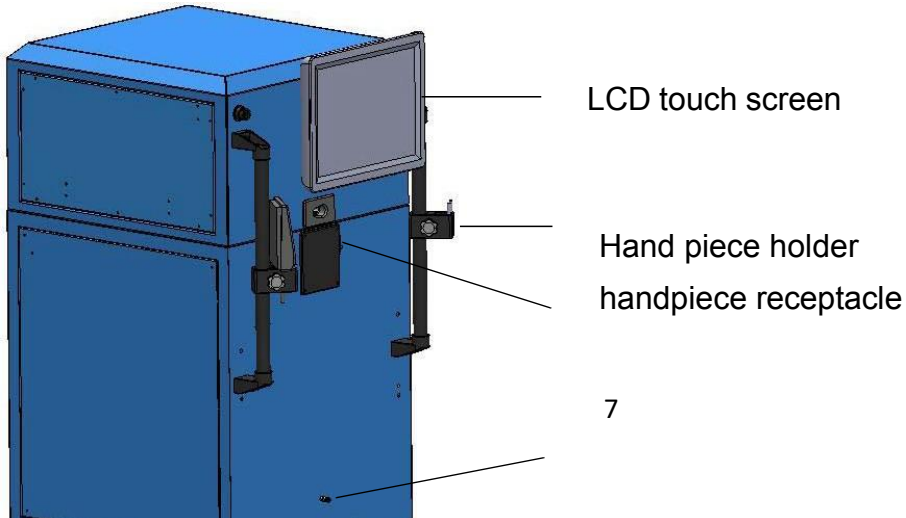
The ***Pulse TX System*** consists of an electrical pulse generator (similar to devices used to electro-coagulate tissue) combined with a hand-piece which is held by the clinician during application of pulses to the skin surface. The hand-piece is coupled to a sterile, single patient-use treatment tip.The tip has an array of pins no greater than 1.27 mm in diameter (18 gauge) which penetrate the skin between 1 and 3 mm. Power settings within a minimum and maximum range are available for selection by the physician, and are software limited to defined setting for each treatment tip by the sponsor. Once the electrical pulse generator is turned on and a power setting is selected, a foot switch triggers a sequence of pre-programmed electrical pulses to an area of skin directly beneath the treatment tip.

A common commercially available sterile coupling gel is applied to the skin or treatment tip surface to ensure good electrical coupling to the tissue.

The three system components are as follows:

1. **Pulse Generator** including a built-in touch screen for setting selection, a power cord, and a mandatory foot pedal for activation of a pulsing sequence
2. **Hand-piece** (re-usable)
3. Single patient-use **Tip** (five different versions available, described below)

Pulse TX Graphic



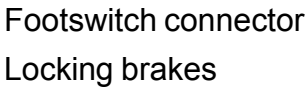


Figure 1. Pulse generator

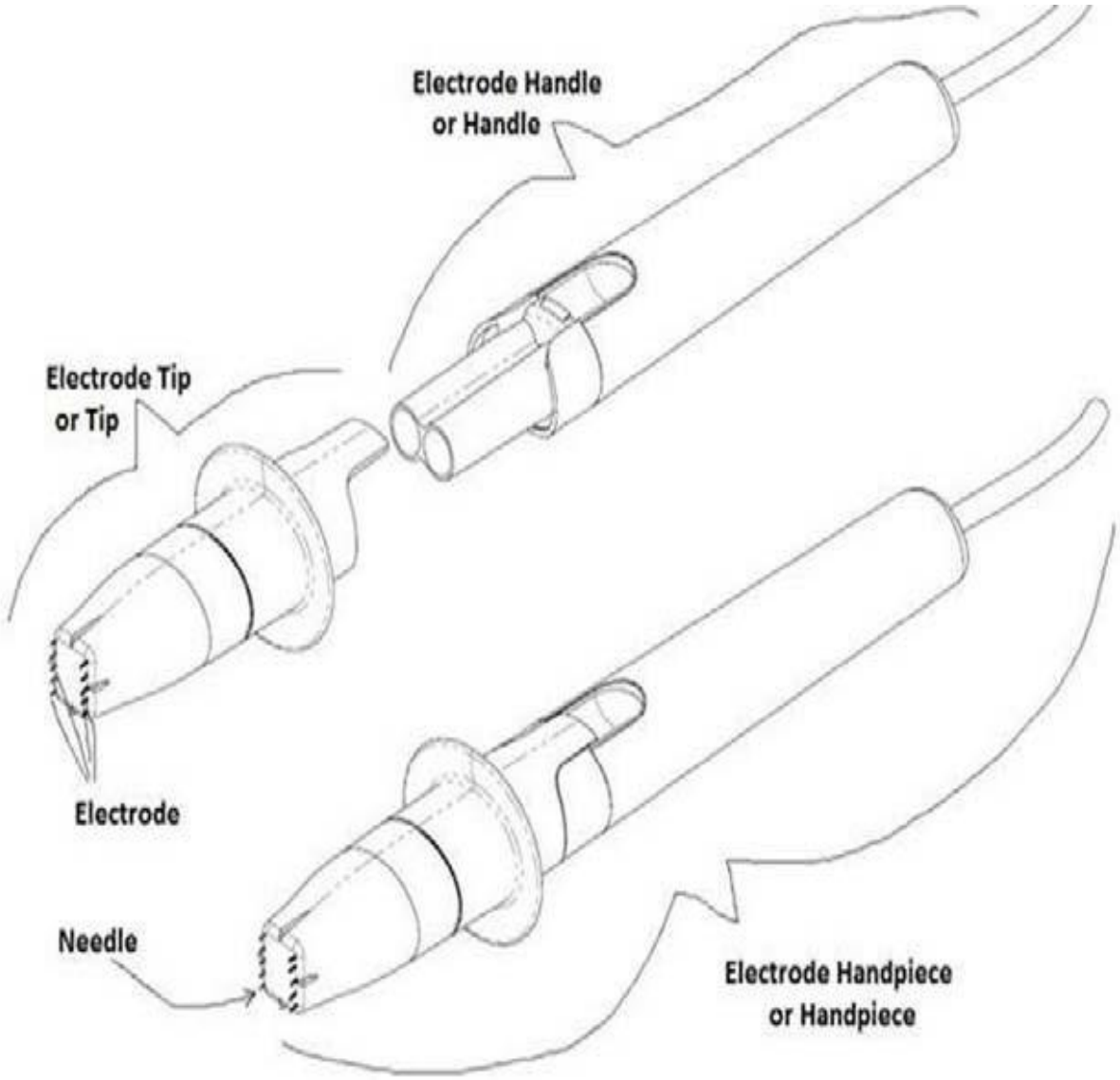
Electrode Handpiece

A reusable handpiece allows the operator to connect the single-use patient treatment tip to the generator. One end of the handpiece connects to the generator; the other end connects to the treatment tip. The handpiece can be cleaned and after each use.

Single-use Treatment Tip

A sterile single-use patient Treatment Tip attaches to the hand-piece. The tip delivers the nano pulses to the area to be treated through an array of micro-needles. Five tip sizes are available:

- 2.5 mm x 2.5 mm Treatment tip
- 5 mm x 5 mm Treatment Tip
- 7.5 x 7.5 mm Treatment Tip
- 10 mm x 10 mm Treatment Tip


Electrode Handle
or Handle
Electrode Tip
or Tip
Electrode
Needle
Electrode Handpiece
or Handpiece

SITE/INVESTIGATOR SELECTION

Up to 5 study sites will be selected to participate in the study. Two of the five sites have been identified. The additional sites have not yet been determined. The table below provides additional information

NAME OF STUDY SITE	**ADDRESS OF STUDY SITE**
Site 1: Aesthetic Artistry Surgical and Medical Center or AASMC	2220 E Bidwell St Folsom, CA 95630 T 916-983-9895
David Kaufman, MD Michele Martinez, RN	2220 E Bidwell St Folsom, CA 95630 T 916-983-9895
Site 2: Aesthetic Center James Newman, MD Melba Herrera, MD	1795 El Camino Real Palo Alto, CA 94306 T 650 321 3223
Same Investigators at alternate location	316 South Eldorado Street, Suite 105 San Mateo, CA650-340-7200 T 650 321 3223
TBD	
NAME OF STUDY MONITOR	
Pamela M. Buckman, MSN Consultant, Pulse Biosciences	2800 Pleasant Hill Rd., Suite 175 Pleasant Hill, CA 94523 T 925 980 7007 F 925 705 7381 pmbuckman@gmail.com

Site #1 above will be the first to initiate the study. This site and the Investigators have been approved by BioMed for a study that is currently open but not active. The required forms and information about subsequent sites and Investigators will be provided to BioMed prior to any study related activities at the site in questions. Prior to enrolling a Subject at any particular site the investigator and appropriate members of the study team at that site will participate in a comprehensive training program designed by Pulse.

SUBJECT RECRUITMENT AND SELECTION

Convenience sampling will be used to enroll subjects exclusively from the patient population at each of the sites. The planned enrollment is set at up to 15 subjects at each site for a total of 60. Recruitment will be conducted via direct communication between potential Subjects with each PI and/or the designated staff.

The invitation to participate will be extended to those patients who are known or present with SK lesions that they individually choose to have removed. The Informed Consent discussion and signature process will be conducted by the PI and/or the designated staff. Once consented, each Subject will be assigned a unique Study Identification Number. See Attachment D for statistical discussion and analysis of proposed Subject population.

Recruitment Statement

If recruitment is not adequate to meet the study requirements, a counter card or other media format may be used. See Attachment D for a proposed recruitment statement. The study **Inclusion** and **Exclusion** criteria are noted below.

Inclusion Criteria:

- Willing to sign the informed consent
- Males or females
- At least 18 years of age but less than 65 years of age
- Has a clinical diagnosis of stable, clinically typical seborrheic keratosis
- Medically determined candidate for at least 4 off-face SK lesions
- Must have at least four treatable SK's and be willing to have Pulse TX treatments on any three of the four treatable SK's. Treatable SK's must:
    - be at least 1 mm in height, and no greater than 3 mm in height.
    - not have a dimension perpendicular to the longest dimension of greater than 7.5mm or longer in any dimension than 20mm..
- Willing to have three of the designated SK's treated in a single treatment session
- Willing to return to the PI's office for five additional study visits at specified intervals over 106 days
- Agrees to high resolution photos of both the treated SK lesions and the untreated SK lesion
- No subject identity will be possible via the "lesion-only" photograph
- Has no evidence of active infection in the designated tissue prior to treatment

- Is not allergic to Lidocaine or Lidocaine-like products
- Not pregnant or lactating
- In the investigators' opinion, be in good general health and unlikely subject to risks from treatment

Exclusion Criteria:

- Implantable electronic devices. e.g., automatic defibrillator
- Active infection or history of infection within 90 previous days in designated test area
- Not willing or able to sign the Informed Consent
- Non English speaking or reading
- Is known to be immune-compromised
- Known to be keloid producer
- On blood thinning medications
- Diseases, conditions, or situations wherein PI judges that the patient is not appropriate for participation in the study

NON SIGNIFICANT RISK STATEMENT

The proposed study fits the criteria for a non-significant designation for the following reasons:

- Subjects will be recruited for the study strictly on a voluntary basis
- Participation in the study takes approximately 1 hour for the first treatment visit and 30 minutes for each of the five post treatment evaluation visits, which includes photographs of both treated and control lesion(s)
- Localized anesthesia will be used to control discomfort at the time of the test device application.
- After the test device is deployed and the anesthesia is dissipated, there may be mild, localized discomfort at the site of the treatment. A small scab or crust may develop. This occurrence is transient and should resolve without intervention.
- Transient and minor discomfort at the treated site and appearance of a small scab typically occurs when other standard treatments are used, i.e., cryogen to remove SK's. Use of the Pulse TX does not raise any additional concerns compared to current methods of lesion removal
- There is no loss of privacy as no study records will be viewed or retrieved by anyone other than the study team members.

- Protected information will not be captured
- Subjects are protected by a coding system to de-identify them

STUDY PROCEDURE

The following is an example of the Subject related activities to be completed for each Subject in the study. The exact order may differ depending on Subject/PI schedules and preferences.

Activities prior to or on same day as study enrollment and occur on same day as treatment visit:

- evaluation for Inclusion/Exclusion criteria
- sign the consent form prior to any study activities
- receive a copy of the signed consent form

Activities on 1st Study Visit Day

- Four SK's are selected that meet the study criteria for treatable SK's. If more than four lesions are present, the patient can state a preference for which 4 lesions are included in the study
- Photographs of each of the four selected SK's will be taken prior to anesthesia or treatment
- Local anesthesia will be applied to the four selected SK's.
- Using a randomization method, exactly three of the selected SK's will be designated to be treated and one will be designated as an untreated control
- The test device will be deployed to the selected SK's according to the randomized order
- a light bandage will be applied
- Subject will be discharged

Activities on 2nd, 3rd, 4th, 5th and 6th Study Visit Days

- Photographs of each of the 3 treated lesions and one non-treated lesion will be taken
- Each lesion will be clinically assessed and the appearance will be rated according to specified criteria.
- Any adverse events will be identified and documented.

RANGE OF DAYS FOR STUDY VISIT INTERVALS		
STUDY VISITS	**TARGET VISIT DATES**	**VISIT DATE RANGES**
1	Treatment Day	NA
2	1 week post TX	7 to 9 days post TX
3	1 month post TX	28 to 35 days post TX
4	60 days post TX	50 to 60 days post TX
5	90 post TX	80 to 90 days post TX
6	106 days post	100 to 111 days post

SUBJECT COMPENSATION

Each subject will be compensated for any inconvenience that study participation may represent. Specifically, each patient will make 6 total visits to the surgeon's office (approximately 4 hrs. total time). In return for this time commitment, the patient will receive $200 for the treatment visit, $100 for each of the 5 follow up visits and $400 bonus for completing all of the scheduled visits. The total amount of compensation will be $1,100.

STUDY BENEFITS

A volunteer for the proposed study is someone who has elected removal of benign lesion(s). Such lesions are typically removed via cryogen or a scalpel. Participation in the study offers another technique to accomplish the same thing as the typical tools. Experience with the Pulse TX to date offers some confidence that the lesions will be successfully removed although no assurances will be offered. The information learned may contribute to the ultimate use of a more safe and effective device available to ablate tissue in future patients.

STUDY/DEVICE RISKS

The Investigators for the study are experienced physicians. Use of the Pulse TX System poses very minimal risk, including scabbing, minor skin pigment changes or scarring and minor discomfort which can be further minimized by covering the spot with a small bandage. Typical wound complications such as infection, bleeding and discomfort which requires prescription medication for relief are not anticipated. If they should occur they would be considered an unanticipated adverse event and managed as such. If the Subject has any questions or concerns about their medical condition or if an unforeseen event should occur the subject will be advised to contact the PIs or their designate.

SITE TRAINING PLANS

Training to be conducted can be divided into two pertinent categories. First is training on use of the Pulse TX System from a technical and clinical perspective. The PIs and their designated assistants will be instructed in aspects of set up, application, management and maintenance of the Pulse TX System. Those persons conducting the training will be Pulse Bioscience technical experts in this area of expertise. A select group of these experts will be present during the testing sessions to provide support as needed. Training will include a dry run. The second category is training in the clinical study requirements and processes. The study team members at the Study Site will be trained via the pertinent documents and files as well as the planned logistics to perform the study according to the protocol and applicable IRS, GCP and Pulse requirements.

INSTITUTIONAL REVIEW BOARD OVERSIGHT

The following Institutional Review Board will be asked to review and approve the proposed study:

Biomedical Research Institute of America
P.O. Box 600870
San Diego, CA 92160
T - 619 282 9997
F - 619 282 9998

If the Subject has any questions or concerns about the study process, they will be advised to contact Biomed at 619-282-9997

STUDY MANAGEMENT AND MONITORING PLAN

Pulse Biosciences, and/or their designated representatives are solely responsible for the proposed study. Pulse will take the necessary steps to assure that the study is conducted in accordance with all regulating authorities as well as applicable Standard Operating Procedures. The Study Monitor will oversee the conduct of the study on a continual basis. Minimal Monitoring visits will be conducted at the following intervals: Site Qualification Visit, Study Initiation Visit, Interim Study Visit and Study Close-Out Visit.

ATTACHMENT A

ARTICLE REPRINT

NUCCITELLI
ARTICLE.pdf

ATTACHMENT B

HISTOLOGY REPORT

A Summary Histology Report of a Dose Ranging Study of Nano-Pulsed Cell Stimulation in a Pre-Excised Human Tissue Model

Microscopic samples of abdominal tissue from seven subjects treated with a novel non-thermal energy device that delivers short low energy pulses to a controlled tissue volume have been reviewed in a detailed histologic analysis. The treatment intervals of the targeted normal abdominal tissue ranged from 10 hours to 60 days prior to a previously scheduled abdominoplasty procedure in which the treated tissue was demarcated six months prior to excision. Six treatment energy levels are reviewed per patient per time interval. Various tissue staining techniques intended to identify specific cellular changes and tissue morphology were utilized in the histologic analysis to characterize the initial tissue responses and the subsequent recovery processes, and sequential findings for each of the six energy levels were compared to normal control punch biopsies in the same patient.

Epidermal Changes: Review of the changes of the entire thickness of the epidermal/dermal layer and subcutaneous fat from day 1 through day 60 was performed. The primary changes due to the non-thermal energy exposure were observed in the treated skin within the epidermal layer of skin. At day one post-treatment, tissue samples from all subjects showed evidence of “ghost cells” in the epidermal layer characterized by intact cell membranes and absence of nuclei within those cells, which indicates a non-viability of those cells. The non-viability of epidermal cells was full-thickness and complete for all energy settings by 1 day post-treatment. In many patients, hair follicles and eccrine glands within the dermal layer of the skin were also visible for histologic review. In specimens in which hair follicles and eccrine ducts were visible, there is partial (50%) to full necrosis of the upper portions of the hair follicles and eccrine ducts. 5 days post-treatment epidermal changes range from 50% to 90% healing (50% - 90% viable epidermal cells). Hair follicles and eccrine ducts when visible in the tissue samples show focal dyskeratotic cells within the hair follicles at 5 and 15 days consistent with partial healing at 5 days and complete recovery by 15 days. Eccrine ducts often showed focal squamous metaplasia, a sign of re-epithelialization. By 15 days the epidermis layer had returned to normal in almost all cases. By 60 days the epidermis, hair follicles, and eccrine glands had all completely returned to normal. Of note in two patients treated at the highest energy level, epidermal necrosis was followed by a formation of an inflammatory eschar. This formation healed by 60 days with some epidermal flattening and minimal papillary dermal fibrosis.

Alterations of dermal collagen: Alterations in the dermal collagen observed were minimal, with no evidence of thermal injury. In several tissue samples exposed to treatment levels five and six, there was focal papillary dermal necrosis evident. This effect was observed at the 1 day and 5 day intervals, but was not observed at the 15-60 day intervals. In two patients at treated at the highest energy level, there was some parallel fibrosis of the dermal collagen bundles at 60 days.

Elastic tissue staining: Elastic tissue remained intact in the vast majority of patients. In treatment levels 4, 5 and 6 there was occasional slight decrease in elastic fiber noted in earlier specimens, at days 1, 5 and 15, with return of elastic fibers observed by days 30 and 60. Taken in summary with the results of the trichrome collagen stain, these findings indicate relative minimal effects on the dermis. This predicts a very low risk of scarring. The risk is greatest in treatment level 6.

Melanocytic density: The number of melanocytes was observed using a MITF immunostain. Staining for melanocytes shows a marked loss of melanocytes in the treatment areas at day one. By day 15 the number of melanocytes return to normal density, comparable to the control specimens. This was observed through 60 days. The rapid return of the melanocytic density to levels comparable to control should be consistent with a relative normalization of skin pigmentation over time

Dermal fibroblasts: The number of dermal fibroblasts appear to decrease at day 1 and day 5. However, by day 15, 30 and 60 the number of dermal fibroblasts was equivalent to pretreatment samples. This suggests a temporary decrease in the number of dermal fibroblasts after treatment and possibly some loss of dermal fibroblasts secondary to the treatment. However, fibroblasts are recruited from normal surrounding skin and peripheral circulation and the population of fibroblasts returned to levels similar to the control in the 15-60 day time period. This finding suggests a recovery of fibroblasts and the associated normal capacity to rebuild connective tissue.

Apoptosis: Measurement of epidermal Caspace staining was performed using immune-histochemical stains to active Caspace 3. This analysis shows no significant expression within the epidermis in the 1 and 5 day post-treatment time frames tested. These findings do not provide clear evidence of apoptotic cell death during the analyzed time frames. Therefore, the precise mechanism for the observation of “ghost cells” and associated cell death was not identifiable by this method.

Inflammation: Overall, the degree of dermal inflammation was minimal compared to other thermal or physical methods of intentionally damaging surface epidermal tissue. There is a small amount of inflammation seen at day 1 and day 5, however the amount of inflammation appears to be sparse and perivascular. Of note, there were several samples which showed focal perivascular inflammation with fibrin deposition suggestive of low grade vascular damage. This can be seen at day 5 through day 60. This appeared to have no clinical correlation to degree of epidermal necrosis or impact on healing.

Summary: The novel method of using low energy nano-pulse cellular stimulation on skin and subcutaneous tissue at six different energy settings was observed to lead to a predictable loss of the epidermal layer of skin at all energy levels by one day posttreatment. The lack of observed effect on dermal collagen suggest that the effect is non-thermal, with a relatively low level of inflammation given the amount of epidermal damage. This lack of inflammatory effect is consistent with preservation of fibroblasts, elastin, and melanocyte recovery. The transient effect on deeper cellular structures in the dermis suggest an affinity of nanopulse energy for highly cellular tissue, and a sparing effect on the less cellular connective tissue of the collagen layer.

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ATTACHMENT C

INDEPENDENT PHOTOGRAPHIC REVIEW

INDEPENDENT PHOTOGRAPHIC REVIEW
STUDY PROTOCOL

BACKGROUND

The NanoPulse System is a medical device intended to create a controlled non-thermal reduction or clearing of targeted tissue areas as an alternative to excisional surgery and other more thermally ablative methods for removing undesired tissue or cells. The system deployed in the study is designed to deliver a timed series of very low energy, high voltage (similar to the voltage of static electricity) pulses of a time length between 100 and 750 nanoseconds (billionths) of a second. The non-thermal effect takes place in a shallow depth of tissue directly below the sterile treatment tip. The device emits significantly less energy than other currently used electro-surgery or electro-cautery equipment.

In several well-designed animal studies, nano-pulsed devices have been demonstrated to reduce or eliminate skin tumors with an acceptable margin of safety. An NSR pilot clinical trial was conducted under IRB oversight beginning in 2012 with a similar version of the Nano Pulse device. A total of ten basal cell carcinomas on the skin of three Subjects were treated with controlled energy settings with a varying number of pulses. Seven of the ten treated areas were free of basal cells at the conclusion of the study, two partially resolved, and one recurred. No scars were visible at the healed sites of any of the successfully treated basal cell carcinomas.

A Human Tissue Study was conducted in late 2016 to early 2017 for the primary purpose of collecting biopsied samples of human tissue that had been tested with controlled exposures to the NanoPulse System at varying energy levels and number of pulses, and followed at 5 standardized time intervals up to 60 days. This single site study enrolled 5 Subjects who were previously identified candidates for abdominoplasty. Each Subject provided 30 treated areas to be biopsied.

A Photographic Review Study was performed including images of 150 non-thermal lesions that were created among the enrolled Subjects. For each of these 150 lesions, specific characteristics associated with normal wound healing were evaluated and rated according to a predetermined scale. This Photographic Review Study was executed according to a comprehensive Protocol including Pre Training and followed by Intra- and Inter-rater analysis. The described Independent Photographic Review Study serves as a model for the proposed Photographic Review SK Study.

PURPOSE of the PHOTOGRAPHIC REVIEW

The independent review of the lesions photographed at each evaluation interval is being conducted to standardize the interpretation of the lesion clearance and appearance over time. The centralized independent review of the photographs can also reduce potential bias in these types of clinical trials where the assessment of the images can be subjective. Ensuring consistency and lack of bias is a critical issue for the Sponsor and FDA. The image acquisition and interpretation protocols help standardize the process across the clinical sites. A central review allows an auditable, rigorous, and uniform process of evaluation, thus achieving higher confidence in the integrity of the data.

OBJECTIVES and SUCCESS CRITERIA

1st objective is to confirm that reported ratings are reproducible over time when considering one reviewer's ratings at two different times so the agreement between the two time intervals should be at least 80%

2nd objective is to confirm that reported ratings are reproducible when considering responses among 3 reviewer's ratings when each is compared to the other two so the agreement between and among the reviews should be at least 80%

REVIEWER SELECTION

Not affiliated with the study

2-3 reviewers qualified by experience with evaluating skin lesions and wound healing

Multiple locations

Independent of each other

PHOTOGRAPH SELECTION & SET UP

- For each lesion in each of the 60 patients, five post-treatment photos of that lesion will be printed in a randomized order, along with an identified baseline photograph of that same lesion. Consistent standard of photographic analysis to select all images to be reviewed at all time points according to uniform criteria
- Images will be masked to time interval, energy settings, size of treatment tip,and specific subject identifiers.

STUDY PROCESS

MATERIALS NEEDED

1. Set of photographs (Training/Reference Set) to be given to each of the reviewers
    - One set of images representing each SK lesion rating option and labeled with the rating it represents
    - One set of images representing each of the skin characteristics to be rated
    - Narrative description of each of the rating options
    - No time intervals displayed
    - No energy settings displayed

2. Set of photographs (Scoring Set) to be given to each of the reviewers
    - Images are restricted to those taken at each of the visit intervals i.e., 7 days, 30 days, 60 days, 90 days and 106 days post initial treatment
    - Images do not include those displayed in Training Set
    - Narrative description of each of the rating options
    - No time intervals displayed
    - No energy settings displayed

3. Set of photographs (V/V Set) to be given to each of the reviewers
    - Redistribute, rearrange images for at least 50% of the randomly chosen images
    - Renumber image identifiers

REVIEWER TRAINING

The training session may be done in person or via use of electronic communication and media formats. Steps to be taken for the training session are as follows:

- Send Training/Reference Set of images to reviewer
- Set up telephone training session (options: Go to Meeting or similar). Should take about 15-30 minutes
- Use consistent trainer for each reviewer session
- Reviewer to keep Training/Reference Set of images to use as reference during the Review session
- Trainer to document time, date, location, attendees, communication format and the topics discussed, etc.

REVIEWER SESSION

The Review session may be done in person or via use of electronic communication and media formats. Steps to be taken for the Review Sessions are as follows:

- Send Scoring Set of images to reviewer
- Set up telephone call (options: Go to Meeting or similar). Should take about 10 minutes to assure understanding of process
- Use consistent contact person for each reviewer call
- Set up deadline date for completion of the review
- Contact person to document time, date, location, attendees, topics covered and communication format.
- Instruct the Reviewer to return the Scored set of images but retain the Training/Reference set

VALIDATION SESSION

The Validation session may be done in person or via use of electronic communication and media formats. Steps to be taken for the Validation Sessions are as follows:

- Send V & V Set of images to Reviewers
- Set up telephone call (options: Go to Meeting or similar). Should take about 10 minutes to assure understanding of process
- Use consistent contact person for each reviewer call
- Set up deadline date for completion of the review
- Contact person to document time, date, location, attendees, topics covered, and communication format.
- Instruct the Reviewer to return all study materials

NOTE: Inter- and intra-rater reliability tests are done to confirm that…

1. a reviewer can look at the same image at two different times and consistently rate the characteristic in both instances. (Intra-rater Reliability) and
2. reviewers are in agreement with each other when they rate the same characteristics under the same circumstances and looking at the same images (Inter-rater Reliability)

DATA MANAGEMENT

All data captured for the Independent Photographic Review Study will be entered into a database specifically designed for this study.

ATTACHMENT D

RECRUITMENT STATEMENT

RECRUITMENT STATEMENT

Are you bothered by small dark spots on your limbs or torso?

Are the spots raised?

Are there at least 4 spots?

Would you like to have them removed?

If you are a good candidate to have the spots removed, you may be eligible to participate in a scientific study.

This clinical study provides financial compensation for study-qualified participants.

For more information about this study, please call our Patient Care Coordinator, xxxxxxx at xxxxxxxxx.

Study participation is limited.